International Journal of Agricultural Science and Research (IJASR)
ISSN (P): 2250-0057; ISSN (E): 2321-0087
Vol. 11, Issue 2, Dec 2021, 227–238

TRANS STELLAR
•Journal Publications •Research Consultancy

A STUDY ON ELECTROPHORESIS ANALYSIS OF BETA-ESTERASE ISOZYMES DURING DIFFERENT DEVELOPMENTAL STAGES OF EVOLVED RACES R_1 AND R_2 OF *BOMBYX MORI* L

MANJULA A. C[1] & KESHAMMA E[2]*

[1]*Department of Sericulture, Maharani Cluster University, Palace Road, Bengaluru, Karnataka, India*

[2]*Department of Biochemistry, Maharani Cluster University, Palace Road, Bengaluru, Karnataka, India*

ABSTRACT

A Science Concerned With Establishing Durable Classification Has Itself Undergone Tremendous Changes In The Last Three Decades. The Traditional Approach Still Is The Basis Of All Taxonomical Studies. The Molecular Data, In Particular, Gel Electrophoresis Of Enzymes And Numerical Methods Of Analysis Have Proven Useful In Many Groups Of Insects And Will See Much Wider Use In Future. Therefore, The Present Study Was Designed With The Main Purpose To Analyze The Activities Of Beta Esterase Isozymes By Electrophoresis Method During Different Developmental Stages Of New Evolved R1 And R2 Races Of Bombyx Mori L. Standardized Disc Electrophoresis Method Was Performed. Esterase Isozymes Form Distinct Enzymes Zones In The Photographs And In The Zymogram And These Have Been Numbered In Cathodal To Anodal Sequence. These Isozyme Patterns Have Been Established After Repeated Runs. The Total Isozymes Of Different Developmental Stages Of Ka, Nb18, Pm, R1 And R2 Have Been Grouped Into Different Zones. The Nomenclature Of Enzyme Banding Pattern Has Been Followed. The Relative Front (Rf) Of The Esterase And Phosphatase Bands Of All The Developmental Stages With Reference To Known Indicator Dye Was Calculated. Results Delineated That 18 Bands In R1 And R2. However, 7 Bands In R1 And 6 Bands In R2 Recorded For Beta Esterase Have Strongly Resulted In R1 And R2. The Esterase Activity Was High In The Pupal Stage Followed By The Larval Stage.

KEYWORDS: Bombyx Mori L, Beta-Esterase, Electrophoresis, Pupa & Larva

Received: Oct 09, 2021; **Accepted**: Oct 29, 2021; **Published**: Nov 08, 2021; **Paper Id.**: IJASRDEC202127

Original Article

INTRODUCTION

A science concerned with establishing durable classification has itself undergone tremendous changes in the last three decades. The traditional approach still is the basis of all taxonomical studies. The molecular data, in particular gel electrophoresis of enzymes, (Yashitake 1963 1968, Eguchi 1965, 1968 Kai and Nishi 1976 Takeda et al., 1992) and numerical methods of analysis have proven useful in many groups of insects and will see much wider use in future (Berlacher 1984).

In recent years and electrophoretic techniques have been extensively used to study enzymatic variations in numerous organisms for various purposes, with reference to the developmental and tissue or organ specific difference of enzymes in Drosophila, silkworm and other insect species. Different methods involving mainly paper, starch, agar gel and disc gel have been employed (Eguchi, 1964, Revanasiddaiah and Chowdaiah 1982).

A number of electrophoretic studies on the esterases have been made in recent years during development (Menzel gal., 1963, Ozita 1962, Lanfer 1961, Revanasiddaiah and Chowdaiah, 1988, Revanasiddaiah et al., 1989, Revanasiddaiah and Manjula, 1992). Eguchi gal. (1964) studied blood esterases of 161 silkworm strains through

agar gel. Nine esterase bands were observed of various tissues of the silkworm Bombyx mori during development. They observed eight bands migrated towards the anode and one towards the cathode. Whittaker and West (1962) studied the insect haemolymph proteins through starch gel electrophoresis. With this scenario, we designed the present study with the main purpose to analyze the activities of beta esterase isozymes by electrophoresis method during different developmental stages of new evolved races *viz.* R_1 and R_2 of *Bombyx mori* L.

MATERIALS AND METHODS

Silkworm Varieties and Rearing

The pure races of bivoltine Kalimpong-A (KA) spinning oval white cocoons, New Bivoltine-18 (NB_{18}) spinning dumbbell white cocoons and multivoltine Pure Mysore (PM) spinning pointed yellow cocoons of mulberry silkworm Bombyx *mori* L. were selected for the present breeding programme. These races were obtained from their respective seed areas and are reared in cytogenetics laboratory, Jnana Bharathi, Bangalore University. The disease free layings were prepared as described by Krishnaswamy, and were incubated at 25°C and relative humidity of 60-70%. On the 8th day composite layings were prepared (10-20 layings were prepared 100-200 eggs were collected from each laying). The hatched worms were reared according to the method described by Krishnaswamy (1978) MS variety of mulberry leaves were used in rearing. The worms were reared in mass up to III instar, after III moult 300 worms were collected in three replicates in order to evaluate the rearing performance. Standard temperature and humidity were maintained in the rearing house.

Breeding

Single and three way crosses were made by using the above said three races. The first single cross involved KA females and PM males. The second single cross involved NB_{18} females and PM males. During the course of breeding, selection was made at the egg, larva, pupa and cocoon stages to fix the desirable traits. F_5 progenies of the respective crosses were back crossed to their respective bivoltine males to improve commercial characters.

Evolutions of New Lines R_1 and R_2

Females of KA and NB_{18} were crossed with males of PM. The composite layings of F_1 hybrid were brushed and reared under standard laboratory conditions. The selection parameters explained earlier were applied to choose the seed cocoons for the preparation of F_2 layings. The replicates showing a higher pupation rate were selected for intra family selection of cocoons. Further, segregation with respect to cocoon colour and built was noticed. Only white oval in case of KAxPM and dumbbell white in case of NB_{18}xPM qualifying the parameter of selection were chosen for breeding in subsequent generations. The females of F_5 were backcrossed to the males of KA and NB_{18} respectively in both the lines and reared up to 11 generations. At the end of the 11th generation, the lines R_1 and R_2 were extracted with higher ERR than their respective better parents, with shorter larval period and with moderate cocoon productivity character in the case of R_1 and R_2.

Breeding Plans I and II													
				I							II		
	KA	O	O	x	PM	Cto		NB18	O	O	x	PM	Cfo
		+	i-						+	+			
				F1							F1		
				F2							F2		
				F3							F3		
				F4							F4		

F5	x	KA	O	↘	er'		F5x	NB18	Cta	+			
				F1							F1		
				F2							F2		
				F3							F3		
				F4							F4		
				F5							F5		
				F6	(R1)						F6	(R2)	

Preparation of Enzyme Extract

The different developmental stages such as 1st day, 5th day and 9th day eggs, five larval instars (I, II, III, IV, and V instars), early, middle and late stages of male and female pupae, male moths before and after copulation. Female moths before and after egg laying of evolved races race-1 (R_1) and Race-2 (R_2) were selected.

Electrophoresis

Disc electrophoresis was performed essentially according to Davis (1964) and Ornstein (1964). A discontinuous gel system consisting of 7.5% lower gel and 3.12 5% spacer gel was used. The lower gel consisted of one part of Tris-hydrochloric acid buffer (36.g Tris+ 48.0 ml of N HCl + 0.46 ml of TEMED, diluted to 100ml. pH 8.9), two parts of cyanogum 41 (3.08 g of cyanogum in 10ml of water), two parts of Ammonium persulphate (140mg of APS in 100ml of water) and three parts of distilled water. 1.2 ml of this solution was poured into clean, dry glass tubes (7 cm x 0.7 cm dia) held vertically. The solution was carefully over layered with distilled water and allowed to photopolymerise for 15 minutes under fluorescent lamp or day light. After polymerisation, the water layer was removed from the top and the spacer gel was added. The spacer gel consisted of 1 part of Tris phophoretic acid buffer (5.7 g tris + 25.6 ml of 1M H_3PO_4 + 0.46 ml of TEMED diluted to 100 ml with distilled water pH 6.9) 2 parts of cyanogen 41 (1.25 g cyanogum 41 in 10ml of Distilled water) 1 part of APS (70 mg in 100 ml) and four parts of water. 0.2 ml of spacer gel was poured on the top of the lower gel each tube layered with a drop of water and allowed to Photopolymerise for 15 minutes. After polymerization, the water was blotted off and the tubes with spacer gel were inserted into the rubber connectors of the upper electrode vessel. The electrode chambers were filled with electrode buffer (0.3 M boric acid and sodium hydroxide buffer pH 8.65). The sample, suitably diluted with 20% sucrose containing bromophenol blue, was carefully layered onto each gel and subjected to electrophoresis in cold (4°C) imposing a current of 2mA per tube for 2 hours.

Staining Procedure

The staining techniques of Ayala et al. (1972) was followed with a slight modification. The stain used for beta esterase constituted 25 mg of beta naphthyl acetate dissolved in 2 of 1:1 acetone water and the same was added to 12.5 ml of 0.1 M phosphate buffer pH 5.9 to which 25 mg of Fast blue RR salt and 12.5 ml of 0.1 M phosphate buffer pH 6.5 were added. The gels were incubated in the stain for 30 minutes until the bands appeared. The gels were then stored in 6% acetic acid.

Esterase isozymes form distinct enzymes zones in the photographs and in the zymogram and these have been numbered in cathodal to anodal sequence. These isozyme patterns have been established after repeated runs. The total isozymes of different developmental stages of KA, NB_{18}, PM, R_1 and R_2 have been grouped into different zones. The nomenclature of enzyme banding pattern has been followed after Ayala et al. (1972). The relative front (Rf) of the esterase and phosphatase bands of all the developmental stages with reference to known indicator dye was calculated as follows:

Rf = (Length gel before staining/ Length gel after staining)

X

(Distance moved by band/Distance moved by marker dye)

RESULTS

The zymograms of R_1 beta-esterase consists of 8 zones. Est-1 zone consists of 2 bands (1 and 2). Band 1 is moderately stained in III instar larvae. Band 2 is absent. Est-2 zone consists of no bands. Est-3 zone consists of 3 bands (5, 6 and 7). Band 5 is faintly stained in female pupae 144h. Band 6 is faintly stained in 24h male pupae. Band 7 is faintly stained in 24h, 120h eggs, 24h in all pupae and female adult after oviposition, moderately stained in III, V instar larvae and darkly stained in IV instar larvae. Est-4 zone consists of 2 bands (8 and 9). Band 8 is faintly stained I instar larvae moderately stained in III instar larvae, 288h female pupae and darkly stained in IV instar larvae. Band 9 is faintly stained in I, V instar larvae, 288h female pupae, female adult before oviposition and moderately stained in 288h male pupae. Est-5 zone consists of 3 bands (10,11 and 12). Band 10 is faintly stained in female adults before oviposition and darkly stained in 120h eggs. Band 11 is faintly stained in 144h male pupae, moderately stained in II instar larvae and darkly stained in 120h eggs, III instar larvae, 24h male pupae, 288h female pupae. Band 12 is moderately stained II instar larvae, darkly stained in 120h eggs, 24h male pupae and 244h female pupae. Est-6 zone consists of 3 bands (13, 14 and 15). Band 13 is faintly stained 144h male pupae, male adult after copulation, darkly stained in 216h egg, IV and V instar larvae, 24h male pupae. Band 14 is faintly stained in male adult before copulation, moderately stained in II instar larvae, female adult before oviposition, and darkly stained in I, IV, V instar larvae, 24h, 144, 288h female pupae. Band 15 is faintly stained in III instar larvae, 24, 144h male pupae, 24, 288h female pupae. Est-7 zone consists of 3 bands (16, 17 and 18). Band 16 is faintly stained in female adults after oviposition, moderately stained in I instar larvae, female adult before oviposition and darkly stained IV, V instar larvae, 24h, 124h male pupae, 144h female pupae and male adults before and after copulation. Band 17 is faintly stained in 288h male pupae and 288h female pupae, moderately stained in I instar larvae and darkly stained in IV and V instar larvae, male adult before and after copulation. Band 18 is faintly stained in 24h male pupae, male adult before and after copulation. Est-8 zone consists of 3 bands (19, 20 and 21). Band 19 is faintly stained 288h male pupae, moderately stained in 144h male pupae darkly stained in female adults before oviposition. Band 20 is moderately stained in 24h male pupae. Band 21 is commonly present in all developmental stages (Figures 1, 2, and 3).

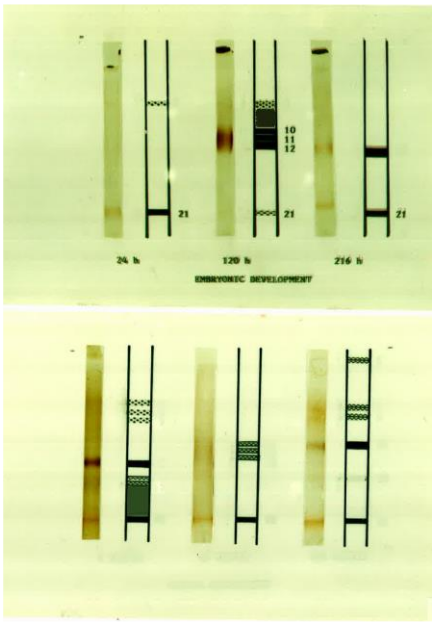

Figure 1: Beta Esterase Zymograms of R_1.

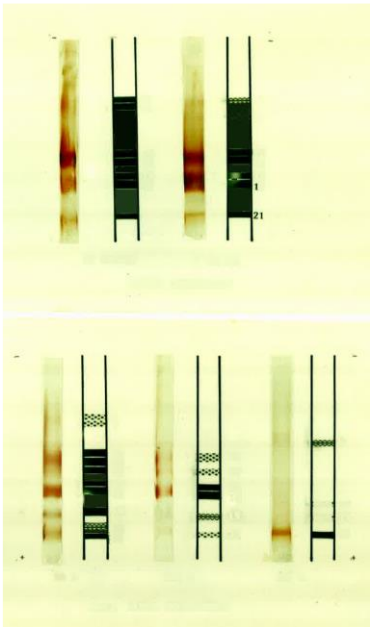

Figure 2: Beta Esterase Zymograms of R_1.

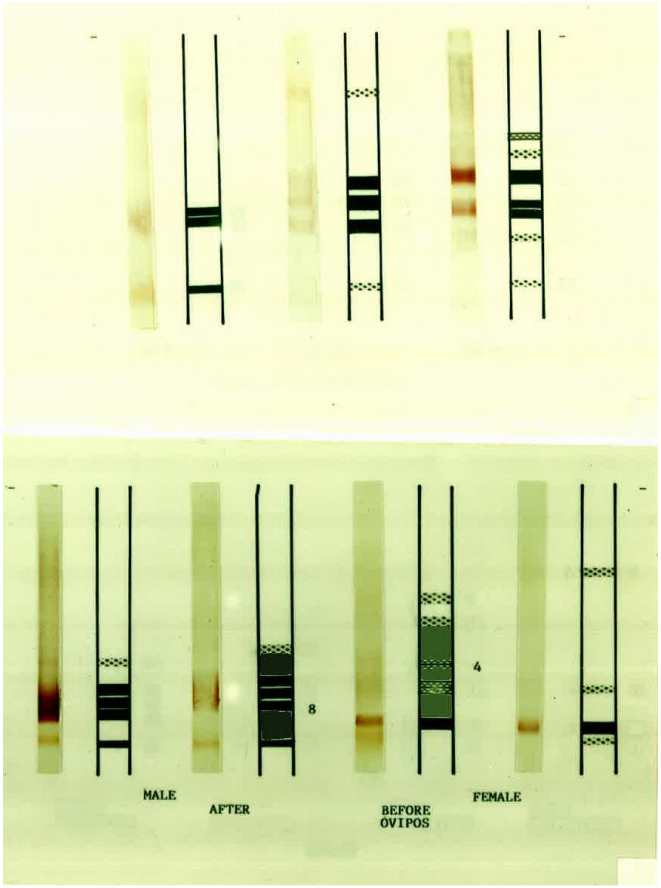

Figure 3: Beta Esterase Zymograms of R_1.

The zymograms of R_2 beta-esterase reveals a total of 8 esterase zones. Est-1 zone does not consist of any band. Est-2 zone consists of 2 bands (3 and 4). Band 3 is faintly stained in 24h male pupae, darkly stained in I instar larvae. Band 4 is moderately stained in I instar larvae and darkly stained in II instar larvae. Est-3 zone consists of 3 bands (5,6 and 7). Band 5 is moderately stained in 144h female pupae, darkly stained in II, III instar larvae, female adult before oviposition. Band 6 is darkly stained in 24h male pupae and female adult before oviposition. Band 7 is moderately stained in II instar larvae and darkly stained in 24h male pupae. Est-4 zone consists of 2 bands (8 and 9). Band 8 is moderately stained in 144h male pupae and darkly stained in 24h male pupae, female adult before and after oviposition. Band 9 is moderately stained in 288h male pupae, darkly stained in 24h male pupae and female adult before and adult oviposition. Band 9 is moderately stained in 288h male pupae, darkly stained in 24h, male pupae, and female adult before and after oviposition. Est-5 zone consists of 3 bands (10, 11 and 12). Band 10 is faintly stained in V instar larvae, moderately stained in male adult before copulation, darkly stained in III, IV instar larvae, 24h and 288h male pupae. Band-11 is faintly stained in V instar larvae darkly stained in IV instar larvae, 24h male pupae, female pupae of 144h. Band 12 is darkly stained in III instar larvae, 288h male pupae, 144h female pupae. Est-6 zone consists of 3 bands (13, 14 and 15). Band 13 is faintly stained in V instar larvae, male larvae, male adult after copulation, moderately stained in IV instar larvae and female adult after oviposition, darkly stained in 144h, 288h male pupae. Band 14 is faintly stained in V instar larvae, male adult before copulation, moderately stained in I instar larvae and darkly stained in IV instar larvae, 144h male pupae, 24h and 288h female pupae. Band 15 is darkly stained in III instar larvae and male adult before copulation. Est-7 zone consists of 3 bands (16, 17 and 18). Band 16 is darkly stained in 216h eggs, V instar larvae, 144h male pupae, 288h female pupae and male adult after

copulation. Band 17 is moderately stained in 24h female pupae and darkly stained in 24h, 216h eggs, 144h male pupae and 288h female pupae. Est-8 zone consists of 3 bands (19,20 and 21). Band 19 is darkly stained in 24h and 216h eggs. Band 20 is darkly stained in 24h, 120h, 216h eggs. Band 21 is common in all the stages (Figure 4, 5, and 6).

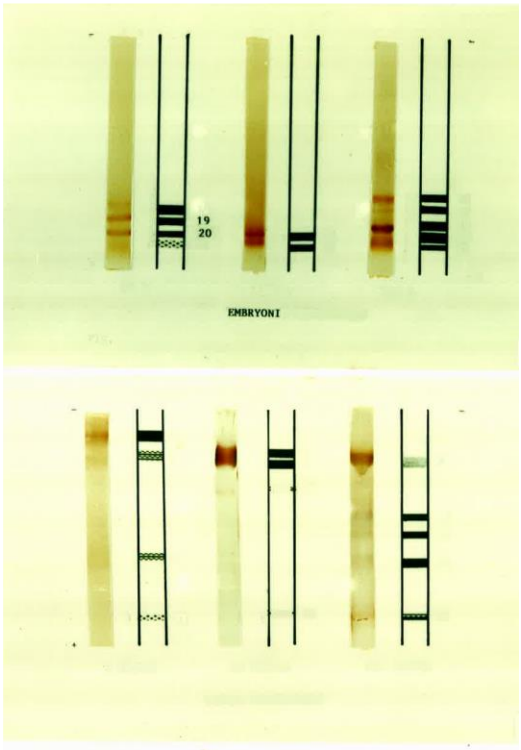

Figure 4: Beta Esterase Zymograms of R_2.

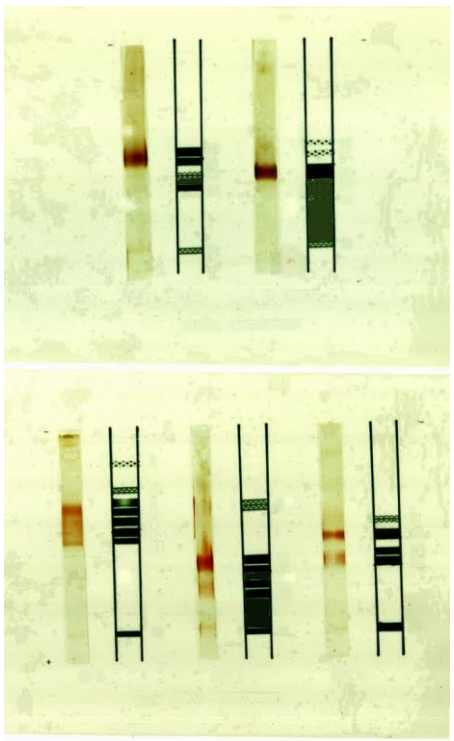

Figure 5: Beta Esterase Zymograms of R_2.

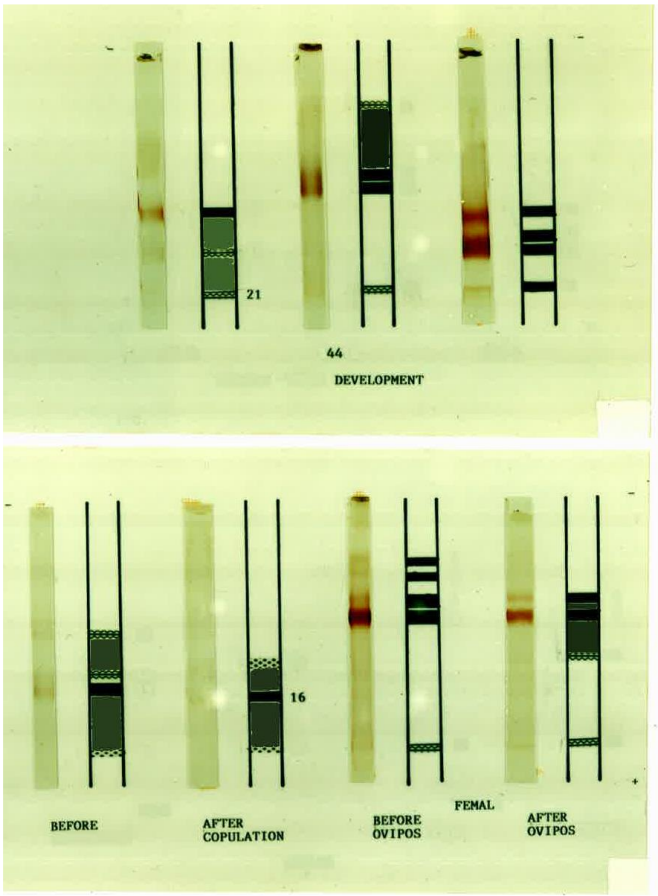

Figure 6: Beta Esterase Zymograms of R_2.

DISCUSSIONS

The changes in beta-esterase zymograms during development revealed 18 bands in R_1 and R_2. However, these bands are not found in any single stage of development of *B. mori*. More or less same investigations have been conducted on the eri silkworm *Philosamia ricini* (Revanasiddaiah et al., 1989), and they reported 30 bands at various developmental stages. In a multivoltine strain of - mori (Pure Mysore) L- and beta- esterase isozyme patterns were reported by Krishnamurthy et al. (1984). But their studies were conducted around egg development and they noticed twelve isozymes in embryogenesis. However, in the present study 7 bands in R_1 and 6 bands in R_2 are recorded for Beta esterase and all the bands have strongly resulted in R_1 and R_2 during embryogenesis. This also agrees with the report of Fei and Sheng (1983). They reported a total of 12 esterase isozymes during embryogenesis.

At larval stages 14, 14, 12, 11 & 12 bands are recorded for beta-esterase. In pupal stages, more number of bands are recorded and in adults, the numbers have been decreased. There is a gradual increase of esterase isozymes from eggs to pupae and they decreased in adults. This variation reflects regulation of gene activity so as to meet the den and of different metabolic activities (Revanasiddaiah et al., 1989, Krishnamurthy al., 1984).

The eggs show high esterase activity. This is due to the presence of large amounts of enzymes stored in the yolk which will be utilized during embryogenesis. The larval, pupal and adult stages reveal maximum heterogeneity like *P. ricini* Esterase isozymes show sexual dimorphism in both pupal and adult stages. This shows stable difference in the expression of different genes in the same race of *B. mori* during ontogeny. Thus the analysis of isozymes different

developmental stages of pure races and their isolated races suggest that the enzyme bands appear, disappear and reappear in different developmental stages. On the basis of their manifestation, the bands have been classified into two categories.

One category persists throughout the developmental stages and called "generalized segregating and non-specific esterases" which include Est-3, Est-4, Est-5, Est-6 zones of Beta esterase. These are comparable to the results found in Drosophila immigrants (Pautelouris and Downer, 1969). The other category which is confined to some of the developmental stages is called "specific non-segregating esterases" which include Est-1, Est-2, Est-7 and Est-8 of beta esterases. These are comparable to the ones found in Zaprionus paravittiger described by Kaur and Parkash (1979).

The developmental esterases show a gradual increase in the number of isozymes from I larval to V larval instar of all the races studied including R_1 and R_2. Such an increase during development has been reported in Drosophila nastuta by Siddaveeregowda et al. (1977) and also insects in general by Laufer (1961). A comparative study pertaining to larval developmental stages of the races show a gradual increase in the number of isozymes from I larval to V larval instar in all the races. This may be due to the voltinism.

The results of the esterase activity in pupal stage of the two races studied indicate that the beta specific esterases the activity is expressed only in female pupae of R_1 the activity is expressed whereas it is absent in all the races but non-specific esterase activity is found in all the races. A comparison of the zymograms of adults of all races indicate the activity of esterase, in general, is lower than that of larval instars and pupae. This may be because all the fatty acids are used up in histogenesis.

The functions of esterase isozymes in the physiology of silkworms have been reported by many workers (Kai and Hasegawa 1972, Pant and Gupta 1980, Oberlander and Schaeiderman 1966, Kaur and Prakash 1979) that esterases help in breaking down lipids and fatty acids. The specific esterases found in various developmental stages could be involved in modifying hormones responsible for subsequently metamorphic events growth, moulting, pupation and differentiation into adult (Kaur and Prakash 1979).

CONCLUSIONS

In conclusion, beta-esterase zymograms during development revealed 18 bands in R_1 and R_2. However, 7 bands in R_1 and 6 bands in R_2 recorded for Beta esterase have strongly resulted in R_1 and R_2. The esterase activity is high in the pupal stage followed by the larval stage. The present findings of beta esterases during antogeny are known to exhibit a vast array of variability implies divergence in the molecular properties of protein and the degree of variability in enzyme activity is a reflection of differential gene action in the genetic programming imprinted by part of the genome to be chronologically expressed or suppressed during the development.

REFERENCES

1. *Yoshitake, N. 1963. On the esterase types in the midgut of the silkworm Bombyx mori L. J. Sericult. Sci., Tokyo, 32: 285-291.*
2. *Yoshitake, N. 1968. Phylogenetic aspects on the Japanese race of silkworm Bombyx mori L. J. Sci. Japan, 37: 83-87.*
3. *Eguchi, M. and Sugimoto, T. 1965. Changes in esterase zymograms in the silkworm, Bombyx mori L. during development J. Insect. Physiol. 11: 1145-1149.*
4. *Eguchi, M. and Yoshitake, N. 1968. Electrophoretic variation of protenase in the digestive juice of the silkworm, Bombyx mori L. Nature, 214: 843-844.*

5. *Kai, H. and Nishi, K. 1976. Diapause development of eggs in relation to esterase A activity. J. Physio. 22: 133-142.Bombyx Insect.*
6. *Takeda, S., Azuma, M. and Eguchi, M. 1992.Alkaline phosphatase isozymes of the silkworm midgut polymorphism in the enzyme activity antigen and electrophoretogram. J. Serie. Sci. 59(2): 127-134.*
7. *Berlocher, S.H. 1984. Insect molecular systematics. Ann.Rev. Entomol. 29: 403-428.*
8. *Eguchi, M., Yoshitake, N. and Kai, H. 1964. Types and silkworm inheritance of the blood esterase in the Bombyx mori L. Jap. J. Genet.*
9. *Revanasiddaiah, H.M. and Chowdaiah, B.N. 1982.Electrophoretic studies on protein changes during developmental stages of Anopheles stephensi. Indian J. Med. Res. 75: 40-44.*
10. *Menzel, D.B., Craig, R., Hosking, W.M. 1963. Electrophoretic properties of Esterases from susceptible and resistant strains of the housefly Musca dornestica L. J. Insect Physiol. 9: 479-493.*
11. *Ozita, Z. 1962 Genetico-biochemical analysis on the enzyme activities in the housefly by gel agar electrophoresis. Jap. J. Genet. 37: 518-521.*
12. *Laufer, H. 1961. Forms of enzymes in insect development. Ann. N.Y. Acad. Sci. 94: 825-835.*
13. *Aggarwal, Anupriya, and Praveen Kanth. "DNA encryption." International Journal of Computer Science and Engineering (IJCSE) 3.3 (2014): 51-66.*
14. *Revanasiddaiah, H.M. and Chowdaiah, B.N. 1988. Comparison of esterase isozyme patterns during the developmental stages in wild and black mutant of Anopheles stephensi. Proc. Int. Con. Medic. Veta. Dipt. Ceske Budejovice. Czechoslovakia. 81-86.*
15. *Revanasiddaiah, H.M., Geetha, R. and Mukthamba, H.M. 1989.Electrophoretic patterns of esterase isozymes during the developmental stages of non-mulberry Esi siworm. Philosomia ricini. Proc. Conf. on Cytol. and Gent. 2: 242-249.*
16. *Al-Mussawi, Abdulameer Abdullah. "New report of some bacterial species isolated from human sputum among patients with respiratory tract infections using 16srdna sequencing." International Journal of Applied and Natural Sciences (IJANS) 4.3 (2015): 65-74.*
17. *Revanasiddaiah, H.M. and A.C. Manjula, 1992a. Evolution of superior v riety of Bombyx mori L. I. Hybridization and selection. Proc. Conf. on Cytol. and Gent. 3 172-176.*
18. *Whittaker, J.R. and West, A.S. 1962. A starch gel electrophoretic study of insect haemolymph proteins. Canad. J. Zool. 40: 665-671.*
19. *Krishnaswamy, S. 1978. Improved rearing, CSB Publication.*
20. *Davis, B.J. 1964.Disc electrophoresis. II. Methods applications to human serum protein. Ann. N.Y. Sci., 121: 404-412.*
21. *Ornstein, L. 1964. Disc Electrophoresis-I method application to human serum proteins. Ann. N.Y. Sci. 121: 321-349.*
22. *Bedjargi, Pruthvi RAJ C., and RS Kulkarni. "SDS-Page Analysis and Electron Microscopy of Corpuscles of Stannius Secretion in the Freshwater Fish, Notopterus Notopterus." International Journal of General Medicine and Pharmacy (IJGMP) 3 (2014): 1-6.*
23. *Ayala, F.J, Powell, J.R., Tracey, M.L., Mourao, C.A. and Sales, S.P. 1972. Enzyme variability in Drosophila willistoni group IV. Genie variation in natural population of Drosophila wilstoni. Genetics. 70: 113- 121.*
24. *Krishnamurthy, N.B., Ramesh, S.R. and Rajasekara Setty, M.R.1984. Developmental profiles int he isozyrnes of land B. Esterases in the ernbryogenesis of silkworm Bornbyx rnori. Current Science, Vol. 53, 281-284.*

25. *Seong, S.I., Kobayashi, M. and Yoshitake, N. 1983. Activities of acid phoshpatase and nucleases during metamorphosis in the midgut of the silkworm Bombyx mori. J. Serie. Sci. Jpn. 52: 191-197.*

26. *Pantelouris, E.N. and Downer, R.G.H. 1969. J. Insect. Physiol. In: India. J. Exp. Biol., 1978, 16: 546 by Prakash and Reddy (Vol. 15: 2357).*

27. *Kaur, P. and Prakahh, R. 1979. Ontogenic esterase isozymes and their significance in Zaprionus paravittiger. Indian J. Exp. Biol., 17: 644.*

28. *Siddaveere Gowda, L., Rajashekara Setty, M.R. and Ranganath, H.A. 1977. Ontogenetic changes in the alpha and beta esterases in Drosophila nasuta*

29. *. First All India Cell Biology Conference, Varanasi, 22.*

30. *Kai, H. and Hasegava, K. 1972. Electrophoretic protein patterns and esterase zymograms in ovaries and mature eggs of B. mori in relation to diapause J. Insect. Physiol .1972, Vol. 18, pp. 133-142.*

31. *Pant and Gupta, 1980. Current. Sci. 49: 344-346.*

32. *Oberlander, H. and Schariderman, K. 1966. Nature, 212: 432-433.*